end result of this PCR reaction will be a segment of dsDNA, from primer 1 to primer 2 [essentially forward and reverse] with one specific end designed to be captured onto a medium and the opposite end designed for detection. The method is intrinsic and simultaneous and lends itself to ease of purification after capture and prior to detection and subsequent quantification. This procedure could easily lend itself to the detection and quantification of HIV in a patient or as a low cost method for screening the blood supply. Other uses would be in detection and quantification of cancer genes in the form of their messenger RNA. Other uses for diagnostics would be multiplex screening of pathogenic viruses as well as a low cost viral load for Hepatitis B and C.

The following is an example of how this procedure may be used to detect an HIV viral load. Intravenously drawn blood is collected and 1 milliliter is diluted into a solution of RNA preservative solution. The RNA is purified out of the solution by the usual biochemical protocols with the final step eluting from a silica spin column. The RNA is eluted with RNA free water, 40 micro liters final volume, and placed into a PCR reaction tube. To this solution will be added 20 units AMV Reverse Transcriptase, 10X buffer [suitable for use with the particular PCR enzymes], and 3 units of Hot Start DNA Polymerase, 25 micromoles each of dUTP, dATP, dCTP, & dGTP. The primers are labeled as follows and are constructed in a 5'

to 3′ direction suitable for RT-PCR of HIV. HIV GAG Forward FAM [5′]-ATAATCCACCTAATCCCAGTAGGAGAAAT- [3′], mp= 78C, and GAG Reverse BIOTIN [5′]-TTTGGTCCTTGTCTTATGTCCAGAATG with a mp= 76C and were added to the reaction at a level of one micro molar. All of the reagents that are added to the RNA can be stored as a concentrate solution so as to impart an automated ability to the assay. An arbitrary 10 micro liters of reagent could be added to the 40 micro liters of RNA to make a 50 micro liter RT-PCR reaction. The reaction conditions are as follows: the Reverse Transcription step is allowed to occur over the course of one hour with a starting temperature of 45C and reaching a final temperature of 65C at an increase of 1 C per 3 minute, the "Hot Start" is allowed to proceed at 95 C for 15 minutes to liberate the DNA Polymerase activity, and then the following temperatures will be maintained for 40 cycles [1] 94 C for 30 seconds, [2] 55 C for 30 seconds, & [3] 72 C for 30 seconds. After the PCR, the reactants are pipetted into a strepavidin-coated plate suitable for use with a 96 well fluorometer. The binding reaction of Biotin to strepavidin is allowed to occur for a minimum of 30 minutes. The well is rinsed 3 times with Tween Tris Buffered Saline for three times [this step can be done manually or automatically]. The plate is then read for the amount of fluorescence and this should be directly proportional to the amount of virus isolated. In this particular reaction, FAM will be a 5′ labeled Flourescein for the detection of fluorescence and the Biotin, 18-space

linker, will be used for capturing the Amplicons onto a strepavidin coated plate. It does not matter whether the forward or reverse primer gets the capture or detection agent as the Amplicons will still have the ability to be read and their construction is not critical. The ability of the Amplicons to be simultaneously captured, washed, and subsequently detected is what is novel about the modifications of this reaction and analysis mechanism. Although this procedure does NOT lend itself to a real-time assay, it does NOT require the expensive apparatus either. The design of this assay lends itself to automation for high throughput screening at a relatively low cost with reagents readily available.